REMARKS

Claims 56-61, 64-68, 84-93, and 96-103 are currently pending in the application.

Claims 101-103 have been newly added to more fully encompass Applicants' invention. Claims 101-103 are counterparts to currently pending claims 87, 92, and 93.

Claims 56, 58-61, 84, and 85 have been amended to correct the inadvertent omission of the micron symbol ("μ") from the previous listing of the claims.

Claims 57, 67, 68, and 100 have been amended to clarify the attachment of nucleic acids "onto beads" (see, *inter alia*, the originally filed application at page 31, lines 21-29: "...In still further embodiments, one or more of the reagents used in the sequencing reactions is delivered on beads"; page 39, lines 21-22: "In these early studies, sequencing of a PCR product using streptavidin-coated magnetic beads as a solid support was presented"; and original claims 84-87: "...the analytes anchored to the plurality of microparticles are exposed to the reagents..." and "...wherein said analyte is DNA").

Claims 65 and 66 have been amended to clarify the placement of nucleic acids in "discrete regions" (see, *inter alia*, the originally filed application at the abstract: "These methods permit a very large number of independent sequencing reactions to be arrayed in parallel, permitting simultaneous sequencing of a very large number (>10,000) of different oligonucleotides"; page 6, lines 16-23: "A sequence apparatus having 10,000 (or more) sites could, in a single run, determine the mRNA species present at a concentration of as little as 1:10,000 (or less)..."; and page 11, lines 14-20: "The anchor primer can be linked to the solid support to reside on, or within, the solid support. In some embodiments, the plurality of anchor primers is linked to the solid support so that they are spaced at regular intervals within an array...").

New claim 101 recites "wherein the nucleic acid is DNA" (see, *inter alia*, the originally filed application at page 8, lines 26-30 to page 9, lines 1-5: "...Other uses include the sequencing of artificial DNA constructs to confirm or elicit their primary sequence...as well as to obtain the sequence of cDNA from single cells...In addition, the methods allow for the sequencing of PCR products and/or cloned DNA fragments of any size isolated from any source"; page 9, lines 6-13: "The methods of the present invention can be also used for the sequencing of DNA fragments generated by analytical techniques that probe higher order DNA structure by their differential sensitivity to enzymes..."; and original claim 87: "...wherein said analyte is DNA").

New claim 102 recites "wherein said pyrophosphate sequencing reagent is luciferase" (see, *inter alia*, the originally filed application at page 6, lines 5-12: "Preferably, the pyrophosphate is detected by contacting the sequencing byproduct with ATP sulfurylase under conditions sufficient to form ATP...A preferred enzyme for detecting the ATP is luciferase...The reactants and enzymes used herein, e.g, the ATP sulfurylase, luciferase, and apyrase, can be attached to the solid surface"; and page 31, lines 21-29: "In various embodiments, some of the components of the reaction are immobilized, while other components are provided in solution. For example, in some embodiments, the enzymes utilized in the pyrophosphate sequencing reaction (e.g., sulfurylase, luciferase) may be immobilized if desired onto the solid support...In still further embodiments, one more of the reagents used in the sequencing reactions is delivered on beads").

New claim 103 recites "wherein said pyrophosphate sequencing reagent is sulfurylase" (see, *inter alia*, the originally filed application at page 6, lines 5-12: "Preferably, the pyrophosphate is detected by contacting the sequencing byproduct with ATP sulfurylase under conditions sufficient to form ATP...A preferred enzyme for detecting the ATP is luciferase...The reactants and enzymes used herein, e.g, the ATP sulfurylase, luciferase, and apyrase, can be attached to the solid surface"; and page 31, lines 21-29: "In various embodiments, some of the components of the reaction are immobilized, while other components

are provided in solution. For example, in some embodiments, the enzymes utilized in the pyrophosphate sequencing reaction (e.g., sulfurylase, luciferase) may be immobilized if desired onto the solid support...In still further embodiments, one more of the reagents used in the sequencing reactions is delivered on beads").

The amended claims have been included solely to expedite patent prosecution in accordance with the U.S. Patent Office Business Goals (65 Fed. Reg. 54604 (September 8, 2000)). Applicants reserve the right to present any cancelled subject matter in a co-pending application.

These amendments are supported by the application as originally filed, and do not constitute new matter. Specific support for the amendments is shown in parentheses, above. Entry of these amendments is respectfully requested.

Objective evidence of non-obviousness

In addition to the Response and Rule 131 Declaration mailed January 25, 2005, Applicants provide herewith objective evidence of non-obviousness for the instant claims. Objective evidence of non-obviousness must be considered during any analysis under §103. *Graham v. John Deere*, 383 U.S. 1, 17-18 (1966); MPEP §§716.01(a), 2141, and 2144.08. Such "secondary considerations" can be the most probative and cogent proof of non-obviousness on the record. *Stratoflex, Inc. v. Aeroquip Corp.*, 713 F.2d 1530, 1538 (Fed. Cir. 1983). Even where the prior art is found to suggest the claimed invention, evidence of unexpected results and long-felt need can lead to a final decision of non-obviousness. *Simmons Fastener Corp. v. Illinois Tool Works, Inc.*, 739 F.2d 1573, 1575-76 (Fed. Cir. 1984). Applicants' objective evidence must share a nexus with the claims, yet only a reasonable correlation is required. *See* MPEP §§716.01(b) and 2144.08. Thus, unexpected results need not be presented over the entire range of the claims. *See Id.* It is sufficient to show unexpected properties for an exemplary embodiment of the invention. *See, e.g., In re Chupp*, 816 F.2d 643, 646 (Fed. Cir. 1987); MPEP §2144.08.

As evidence of the unexpected results and long-felt need for the claimed invention, Applicants provide articles from *Nature* (Exhibit 1; Margulies et al., "Genome Sequencing in Microfabricated High-Density Picotiter Reactors," *Nature*, Epub July 31, 2005) and the *New York Times* (Exhibit 2; Wade, "2 New Methods to Sequence DNA Promise Vastly Lowered Costs," *New York Times*, August 9, 2005). As evidence of commercial success of the claimed invention, Applicants provide press releases from 454 Life Sciences (Exhibit 3; 454 Life Sciences "454 Life Sciences Receives $11.5 Million in Milestones from Roche," *PRNewswire-FirstCall*, July 26, 2005) and Roche Diagnostics (Exhibit 4; Roche Diagnostics "Roche and 454 Life Sciences Enter Exclusive World-Wide Agreement," *Roche-Media News*, May 12, 2005) as well as the system description from Roche Applied Science (Exhibit 5; Roche Applied Science, "Revolutionize Whole Genome Sequencing," from www.http://www.roche-applied-science.com/fst/products.htm?/sis/sequencing/ genome/announce.htm, 2005).

The *Nature* article details the claimed substrate and apparatus as including:

- A fiber optic slide etched to produced 1.6 million wells (Exhibit 1, page 2, left column; *see instant claims 56 and 84: cavitated fiber optic wafer...at least 10,000 wells...etched into the top surface; and claim 66: wherein said substrate comprises 10^5 or more different groups of nucleic acid sequences in discrete regions*);
- with a fiber diameter of 47 μm (Exhibit 1, page 2, left column; *see instant claims 56 and 84: each individual optical fiber having a diameter between 3 and 100 μm; and claim 85: wherein the diameter of each individual optic fiber in the cavitated wafer is between 6-50 μm*);
- with a well depth of 55 μm (Exhibit 1, page 2, left column; *see instant claims 56 and 84: the depth of each well ranges from between one half the diameter of an individual optical fiber and three times the diameter of an individual optical fiber*);
- with beads attached with genomic DNA (Exhibit 1, Figure 1; *see instant claims 57 and 100: wherein the nucleic acid is immobilized on the wells or on said beads; and claim 87: wherein the nucleic acid is DNA*);

- with beads attached with sulfurylase and luciferase as sequencing enzymes (Exhibit 1, page 5, left column; *see instant claims 56 and 84: said beads having a pyrophosphate sequencing reagent attached thereto; and claims 92 and 93: wherein said pyrophosphate sequencing reagent is luciferase[sulfurylase]*);
- with a flow chamber for holding the fiber optic slide (Exhibit 1, page 2, Figure 2; *see instant claim 84: a flow chamber having disposed therein a cavitated fiber optic wafer*);
- with a fluidic assembly for delivering individual nucleotides (Exhibit 1, page 2, Figure 2; *see instant claim 84: fluid means for delivering additional pyrophosphate sequencing reagents, including sequential delivery of nucleotide triphosphates*); and
- with CCD camera-based image capture (Exhibit 1, page 5, left column; *see instant claim 84: detection means for detecting optical signals from each well; and claim 86: wherein said detection means is a CCD camera*).

The *Nature* article describes Applicants' substrate and apparatus as providing a system for "whole-genome sequencing" (Exhibit 1, page 1, left column). The article notes that the system is capable of generating 47 million bases of sequence information from test fragments (Exhibit 1, page 4, left column). The article points out that the system can be used at 100% accuracy over greater than 400 bases on single reads (Exhibit 1, page 4, right column). The article states that typical runs can be used to generate 25 million bases of sequence information at an estimated accuracy of 99% or higher (Exhibit 1, page 1, right column). The article notes that Sanger sequencing is substantially slower, less efficient, and more expensive (Exhibit 1, page 1, left column and page 4, right column). The *Nature* article emphasizes the need to replace the Sanger method (developed in 1981) for large-scale sequencing (Exhibit 1, page 1, left column).

The *New York Times* article recognizes Applicants' substrate and apparatus as making "giant strides towards the goal of sequencing the human genome so cheaply that it could be done routinely for medical reasons" (Exhibit 2, page 1). The article makes specific reference to Applicants' use of beads attached with DNA, luciferase, and the "light-sensitive chip" which is distinguished from other sequencing approaches (Exhibit 2, page 2). In the article, Dr. Rothberg (Chairman of 454 Life Sciences and inventor for the instant application) points to the exclusive

ability of the system to sequence novel genomes and to re-sequence the human genome at a cost of $1 million (Exhibit 2, page 2). The *Times* article notes that the Human Genome Project completed in 2003 cost approximately $800 million (Exhibit 2, page 2).

The 454 Press Release indicates that Applicants' substrate and apparatus have been licensed by Roche Diagnostics in a $62 million world-wide agreement for promotion, sale, and distribution of 454's Genome Sequencing Systems (Exhibit 3, paragraph 3). The Press Release notes that 454 Life Sciences has already received $11.5 million from Roche Diagnostics in milestones for the commercial release of the Genome Sequencing Systems and reagents (Exhibit 3, paragraphs 1 and 3). The Press Release specifically notes Applicants' "novel instrumentation...[for] high-throughput nucleotide sequencing, with specific application to sequencing of whole genomes" (Exhibit 3, paragraph 4). The 454 Press Release notes that the instrument is capable of producing more than 20 million bases per run, which is 100 times the capacity of other sequencing systems (Exhibit 3, paragraph 5).

The Roche Press release confirms the $62 million licensing agreement with 454 Life Sciences for the promotion, sale, and distribution of 454's Genome Sequencing Systems (Exhibit 4, paragraphs 2 and 5). The Press Release also confirms the systems' ability to produce more than 20 million bases in each sequencing run (Exhibit 4, paragraph 3). This is further corroborated by the system description from Roche Applied Sciences, which also notes the *Nature* article (Exhibit 5, page 1). The Press Release states that Applicants' substrate and apparatus provide a "scalable, ultra-fast, and cost-effective system with applications for whole genome sequencing" (Exhibit 4, paragraph 3). In the Press Release, the CEO for Roche Diagnostics states that "Customer feedback and our own research show that one of the main limitations of today's approaches to sequencing is throughput. This new technology will significantly increase the speed of sequencing, and thereby has the potential to open up many new applications...all over the world" (Exhibit 4, paragraph 4).

Thus, the *Nature* and *Times* articles, press releases, and system description provide objective proofs of non-obviousness of the claimed invention including unexpected results, long-

felt need, and commercial success. These indicia, furthermore, show a sufficient nexus to the claimed invention. As such, even if Chee could be modified with Kain (which Applicants contest), the claimed substrate and apparatus show unexpected efficiency and operating advantages which could not have been derived from these references. *See U.S. v. Adams*, 383 U.S. 39, 50-51 (1966). It is noted that the devices from Chee and Kain have failed to receive similar acknowledgment. In fact, the *Nature* and *Times* articles emphasize the long felt need to replace Sanger sequencing, and neither Chee nor Kain are mentioned as alternatives. *See Graham*, 383 U.S. at 17-18, 29-30, and 35. By comparison, the claimed substrate and apparatus have been recognized as producing arrays which far surpass the Sanger substrates. The superior qualities of Applicants' devices have been advanced by top-tier publications (and editors and reviewers). Few inventions receive this level of public and peer recognition and commercial success. As such, this is compelling evidence for the non-obviousness of Applicants' claims.

The Legal Standard For Obviousness

Applicants have provided herewith objective evidence of non-obviousness as shown in several publications including *Nature* and *The New York Times*. The unexpected results and long-felt need for Applicants' substrate and apparatus have been recognized by peer- and editor-reviewed publications of the highest caliber. The commercial success of Applicants' system is clear from the world-wide, multimillion dollar agreement entered by 454 Life Sciences and Roche Diagnostics. Moreover, the evidence of unexpected results, long-felt need, and commercial success shares a sufficient nexus with the claimed invention. Thus, even if Chee and Kain could be combined to teach or suggest all the elements of the invention (which is still disputed), the surprising and acclaimed results for the claimed method are sufficient to establish non-obviousness. *See In re Albrecht*, 514 F.2d 1389, 1396 (C.C.P.A. 1975); MPEP §2144.08.

As a final consideration, Applicants note that an earlier position held by the Office should not be set in concrete with any rebuttal evidence evaluated only on its "knockdown ability." *See In re Rinehart*, 531 F.2d 1048, 1052 (C.C.P.A. 1976); MPEP §2144.08. The Office

is expected to consider the facts for rebuttal with a fresh eye, and restart analysis under §103. *See In re Piasecki*, 745 F.2d at 1472; *In re Rinehart*, 531 F.2d at 1052. Any finding must rest on evaluation of all the facts in evidence, uninfluenced by any earlier conclusion reached by Office personnel. *See Id.* For at least these reasons, as well as the reasons set forth in the Amendment and Response mailed January 25, 2005, the instant claims cannot be considered obvious over Chee and Kain.

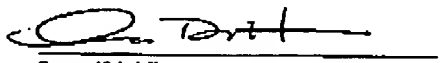
Withdrawal of this rejection is respectfully requested.

CONCLUSION

A favorable action on the merits is respectfully requested. If further discussion of this case is deemed helpful, the Examiner is encouraged to contact the undersigned at the telephone number provided below, and is assured of full cooperation in progressing the instant claims to allowance. While Applicants believe that no additional fees are required, the Commissioner is authorized to charge or credit the undersigned Deposit Account No. 50-0311, Reference No. 21465-501 CIP2, Customer No. 35437, for any additional fees needed.

Dated: October 14, 2005

Respectfully submitted,



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